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PAPER

A therapeutic role for BLyS antagonists

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B lymphocyte stimulator (BLyS) is a vital B cell survival factor. Overexpression of BLyS in mice may lead to systemic lupus erythematosus (SLE)-like disease, and treatment of bona fide SLE mice with BLyS antagonists ameliorates disease progression and enhances survival. BLyS overexpression is common in human SLE, and results from a phase I clinical trial with a BLyS antagonist in human SLE have shown the antagonist to be biologically active and safe. These features collectively point to BLyS as an attractive therapeutic target in human disease. *Lupus* (2004) **13,** 317–322.

Key words: APRIL; B cells; biologic antagonists; BLyS; SLE

Introduction

B lymphocyte stimulator (BLyS; also known as BAFF, TALL-1, THANK, TNFSF13B, and zTNF4) is a 285 amino acid member of the tumor necrosis factor (TNF) ligand superfamily.[1–6] It is expressed as a type II transmembrane protein which is cleaved at the cell surface by a furin protease, resulting in release of a soluble, biologically active 17 kDa molecule.[3,7] BLyS, under physiologic conditions, circulates in trimeric form.[3,8] Some laboratories have, under appropriate *in vitro* conditions, induced BLyS to assemble into virus-like clusters of 60 monomers,[9–11] but other laboratories have not detected multimeric self-assembly.[12,13] Indeed, whether virus-like clusters of BLyS can actually form *in vivo* either in the circulation or locally in tissues remains to be established.

Expression of BLyS is highly restricted to myeloid lineage cells (e.g., monocytes, macrophages, dendritic cells, neutrophils),[1–3,5,7,14] and BLyS mRNA and protein levels are upregulated by interferon (IFN)γ, by interleukin (IL)-10, by IFNα, and by CD40L.[7,14,15] Expression of the three known BLyS receptors (BCMA, TACI, and BAFFR) is also highly restricted. Receptor mRNA expression is largely limited to B cells, although activated T cells may express some TACI mRNA.[16–19] In concordance with the mRNA results, BLyS binds strongly to B cells, weakly (at most) to T cells, and not at all to natural killer (NK) cells or monocytes.[1,20] Most, if not all, of the BLyS that binds to human peripheral blood B cells does so via surface BAFFR and/or TACI, with little, if any, BLyS binding via BCMA.[21] Nevertheless, *in vitro* generated human plasmablasts do upregulate surface BCMA and downregulate surface BAFFR and TACI,[22] so it remains probable that BLyS binds discrete B cell subpopulations *in vivo* via BCMA as well as via BAFFR and/or TACI.

BLyS triggered intracellular signaling is complex. Several TNF receptor associated factors (TRAFs), including TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6, interact with one or more of the three BLyS receptors.[20,23–25] Engagement of BLyS with its receptors activates phospholipase C-γ2[26] and activates both NF-κB1 and NF-κB2 via discrete pathways.[27–29] This culminates in increased B cell survival[22,29–34] which may, at least in part, be secondary to BLyS induced upregulation of BCL-2 and/or BCL-X_L.[31] Indeed, B cells with enforced overexpression of BCL-X_L are protected from the premature death that ensues in the absence of BLyS signaling[35] (see below).

Indispensable role for BLyS–BAFFR interactions in normal B cell development

Mice genetically rendered deficient in BLyS display profound global reductions in mature B cells and in baseline serum Ig levels and Ig responses to T cell dependent (TD) and T cell independent (TI) antigens.[36,37] Remarkably, mice genetically rendered deficient in individual BLyS receptors are highly disparate. BCMA deficient mice exhibit no discernible

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phenotypic or functional abnormalities.[37,38] This suggests that even if the *in vitro* upregulation of BCMA surface expression on human plasmablasts[22] is recapitulated *in vivo* in mice, the remaining surface expression of the other BLyS receptors in BCMA deficient mice is sufficient to transmit the requisite BLyS triggered signals for survival and ultimate function.

TACI is also not critical for the agonist effects of BLyS on B cells. TACI deficient mice harbor *increased*, rather than *decreased*, numbers of B cells (although they do manifest impaired Ig responses to TI, but not TD, antigens).[39,40] As they age, TACI deficient mice develop elevated circulating titers of autoantibodies, Ig deposits in their kidneys with concomitant glomerulonephritis, and premature death.[41] *In vitro* treatment of B cells with anti-TACI monoclonal antibody (mAb) blocks B cell responses to agonists,[41] strongly suggesting that TACI actually transmits a negative signal to B cells.

In contrast to the phenotypes of BCMA or TACI deficient mice, A/WySnJ mice (which bear a mutated *baffr* gene) display deficiencies in mature B cell number and antibody responses reminiscent of (albeit less severe than) BLyS deficient mice.[18,19] [Recently developed BAFFR deficient mice also manifest deficiencies in mature B cells and circulating Ig levels similar to those of BLyS deficient mice (Susan Kalled, BiogenIdec; personal communication).] When injected with exogenous BLyS, A/WySnJ mice do not undergo splenic B lymphocytosis (whereas similarly treated A/J control mice do), and BLyS does not enhance survival of B cells from A/WySnJ mice *in vitro*. Moreover, in bone marrow chimeric mice harboring B cells that bear the mutated *baffr* gene and B cells that bear the wild type *baffr* gene, the B cells bearing the mutated *baffr* gene have decreased *in vivo* survival.[33] Taken together, these observations strongly point to BLyS/BAFFR interactions as the essential ones for the agonist effects of BLyS on B cells. It must be stressed, however, that the entire experience to date with BLyS deficient and BAFFR mutant (and BAFFR deficient) hosts has been in *nonautoimmune prone* mice. Whether BLyS and/or BAFFR play the same indispensable role in B cell development in *autoimmune prone* hosts remains to be determined.

Ig promoting effects of BLyS

In addition to the indispensable role for endogenous BLyS in (normal) B cell development, administration of exogenous BLyS to mice at the time of immunization with antigen results in enhanced *in vivo* antigen specific antibody production.[31] This is due, at least in part, to BLyS mediated inhibition of B cell apoptosis.[22,29–34] Repeated administration of BLyS to mice, even in the absence of intentional antigenic immunization, results in B cell expansion and polyclonal hypergammaglobulinemia.[1] BLyS promotes T cell independent class switching of IgD^+ B cells *in vitro* which, when coupled with crosslinking of B cell surface Ig, leads to secretion of class switched antibodies.[15] Thus, at least some of the Ig promoting properties that BLyS displays *in vivo* may also be T cell independent. Although *in vivo* generation of pathogenic autoantibodies (e.g., anti-dsDNA) in SLE is felt to be a helper T cell dependent process,[42] it remains theoretically possible that production of such autoantibodies could be driven by high levels of BLyS even in the absence (marked reduction) of helper T cell function. This issue warrants further investigation and resolution.

BLyS and its antagonism in murine SLE

Three sets of seminal observations (all in mice) strongly point to a causal relationship between too much BLyS and development of disease. First, constitutive overproduction of BLyS leads not just to polyclonal hypergammaglobulinemia but to elevated titers of multiple autoantibodies (including anti-dsDNA), circulating immune complexes, and renal Ig deposits in some (albeit not all) mice that bear a *blys* transgene (BLyS-Tg mice).[6,43,44] Secondly, SLE prone (NZB × NZW)F1 (BWF1) and MRL-*lpr/lpr* mice harbor elevated circulating levels of BLyS at the onset of disease.[6] Thirdly (and most importantly from the vantage point of a clinician), these SLE prone mice respond clinically (decreased disease progression and improved survival) to treatment with a soluble fusion protein between one of the BLyS receptors (TACI or BAFFR) and IgG Fc (TACI-Ig and BAFFR-Ig respectively).[6,28]

Of note, although the salutary clinical response in BWF1 mice to one BLyS antagonist (BAFFR-Ig) in one study was associated with reduced circulating levels of anti-dsDNA antibodies,[28] the dramatic *in vivo* clinical response in BWF1 mice to a different BLyS antagonist (TACI-Ig) in another study was *not* associated with any reduction in circulating anti-dsDNA titers.[6] It is not known whether these disparate results are due to inherent differences in the nature of the BLyS antagonists used, but, regardless, they do strongly suggest that effective blockade of *clinical autoimmunity* by BLyS antagonists may ensue via an *autoantibody independent* pathway. This intriguing possibility requires additional investigation.

Overexpression of BLyS in human SLE

Cross-sectional studies have demonstrated elevated circulating levels of BLyS in approximately 20–30% of human SLE patients tested at a single point in time.[45,46] Weak correlation was observed between circulating BLyS and total IgG levels, and stronger correlation was observed between circulating BLyS levels and anti-dsDNA titers. This parallels the observations in BLyS-Tg mice that elevations in autoantibody titers are out of proportion to elevations in total serum Ig levels.[6,43,44]

Since cross-sectional studies are silent with regard to duration of an abnormality, we performed a 12 month longitudinal study of 68 SLE patients (and 20 healthy control subjects) in whom we serially measured several parameters, including serum BLyS levels and clinical disease activity.[47] Whereas the control subjects uniformly maintained stable 'normal' serum BLyS levels over time, elevated serum levels of BLyS were persistently observed in approximately 25% of the SLE patients, with intermittent elevations in serum BLyS levels being observed in approximately an additional 25% of patients. Given the inhibitory effects of high dose corticosteroid treatment on circulating BLyS levels,[47] these percentages likely are *underestimates* of the true prevalence of BLyS dysregulation in SLE patients. Although the mechanism underlying BLyS overexpression remains to be determined, it is clear that BLyS overexpression is common (but probably not universal) among human SLE patients.

Of importance, although circulating BLyS levels over time do not overtly correlate with disease activity (measured by SLEDAI) *for any individual SLE patient*,[47] they do correlate over time with disease activity in a large SLE population (245 patients from four different medical centers followed for an average of 15 months) when analysed *in aggregate*.[48] Thus, although BLyS has no known direct proinflammatory properties, its positive effects on B cell survival and/or autoantibody production appear to increase the likelihood of aggravating and/or exacerbating disease.

Therapeutic antagonism of BLyS in human SLE

The associations between levels of circulating BLyS and autoantibodies and clinical disease activity in human SLE, coupled with the success of TACI-Ig and BAFFR-Ig in treating murine SLE,[6,28] have given rise to the widely held belief that BLyS antagonism will play a salutary therapeutic role in human SLE (Table 1). This notion has already prompted the initiation and completion of a phase I clinical trial in SLE patients with a human anti-BLyS mAb.[49,50] A total of 70 patients were enrolled in this multicenter double-blind trial, and each patient received either a single infusion of drug at one of four doses (or placebo) or received two infusions of drug at one of the same four doses (or placebo) separated by three weeks. Biologic activity of the anti-BLyS mAb was documented by a reduction in circulating B cells among drug treated (but not placebo treated) patients, and safety of the anti-BLyS mAb was documented by there being no difference in frequency of adverse events between drug treated and placebo treated patients. In light of the short treatment course (one or two infusions), clinical efficacy, not surprisingly, was not apparent. A phase II clinical trial with this anti-BLyS mAb, powered to detect clinical efficacy, is currently under way.

Table 1 BLyS antagonists under development

Antagonist	*Developing company*	*Antagonist type*	*Clinical status*
LymphoStat-B™	Human Genome Sciences	mAb	Phase I in SLE complete Phase II in SLE initiated
TACI-Ig	ZymoGenetics/ Serono	Fusion protein	Phase I in normals initiated
BAFFR-Ig	Biogen/ Genentech	Fusion protein	Preclinical

In addition to anti-BLyS mAb, other BLyS antagonists are being developed and evaluated for use in humans. These include TACI-Ig, which is currently undergoing phase I evaluation in healthy normal volunteers in the UK, and BAFFR-Ig, which is undergoing preclinical trials in primates. Clinical efficacy of these antagonists is, obviously, also not yet known.

In truth, biologic antagonists directed against BLyS and/or its receptors need not be limited to mAb or receptor fusion proteins. Other attractive candidate biologic antagonists include BLyS analogues that competitively bind to BAFFR but do not trigger signaling as well as BAFFR blocking agents that render the receptor inaccessible to BLyS. Indeed, there is no a priori reason that BLyS antagonists must necessarily be biologic. They could be small molecular weight synthetic compounds as well. In any case, development of appropriate BLyS analogues and/or BAFFR blockers will require more detailed studies of BLyS/BAFFR interactions and how (whether) other cell surface structures affect such interactions.

An intriguing possible means of BLyS antagonism is to let BLyS do the job itself. In addition to its full length isoform, a naturally produced shorter isoform of BLyS (called ΔBAFF) has also been identified.[51] ΔBAFF is biologically inactive, and since it has the capacity to form heterotrimers with full length BLyS, ΔBAFF can

actually block BLyS activity. Whether ΔBAFF production (relative to that of full length BLyS) is altered in SLE, how ΔBAFF production and degradation are regulated, and what determines production of one isoform rather than the other are questions that remain to be addressed. Nevertheless, it remains plausible that very efficient antagonism of the autoimmunogenic effects of BLyS could arise by enhancing production of the ΔBAFF isoform at the expense of the full-length isoform.

APRIL: the 'cousin' of BLyS

As indicated above, treatment of BWF1 or MRL-*lpr/lpr* mice with TACI-Ig resulted in clinical improvement.[6] However, TACI-Ig binds and neutralizes not just BLyS but APRIL as well, a 250 amino acid member of the TNF ligand superfamily that shares substantial homology with BLyS and binds to two of the three BLyS receptors (BCMA and TACI)[52–56] but not to BAFFR.[18] APRIL costimulates B cells *in vitro* and *in vivo*,[15,53,54] albeit with considerably less potency than that of BLyS.[57]

Although TACI-Ig can bind and neutralize both BLyS and APRIL, it is, nevertheless, almost certain that neutralization of BLyS lies at the core of the salutary clinical response. First, administration of BAFFR-Ig, which binds and neutralizes *only* BLyS but neither binds nor neutralizes APRIL, effectively ameliorates disease progression in BWF1 mice.[28] Secondly, APRIL itself, even when overexpressed, has no perceptible autoimmunogenic potential. Although constitutive overexpression of APRIL in APRIL-Tg mice leads to enhanced T cell survival and antigen-specific antibody responses, such mice do *not* develop overt B cell abnormalities or serologic or clinical autoimmunity.[58]

This last point is especially important, since APRIL and BLyS can form heterotrimers which circulate *in vivo*.[59] Although APRIL homotrimers do not bind BAFFR,[18] BAFF/APRIL heterotrimers (BAHT) do bind BAFFR and display BLyS-like biologic activity *in vitro*.[59] How BAHT are formed, what determines BAHT formation versus BLyS (and APRIL) homotrimer formation, and what differential biologic potencies and effects (if any) BAHT and BLyS homotrimers have *in vivo* remain to be established. Although it may be that APRIL overexpression can enhance the autoimmunogenic effects of BLyS overexpression under unique *in vivo* conditions via the formation of BAHT, we believe this to be highly unlikely. First, as indicated above, APRIL-Tg mice have demonstrated that APRIL has little, if any, capacity to promote serologic autoimmunity and/or SLE-like disease.[58] Secondly, in our cohort of 68 SLE patients longitudinally studied over a 12 month period, serum APRIL levels *inversely* correlated with serum anti-dsDNA titers (in anti-dsDNA positive patients) and *inversely* correlated with clinical disease activity (as measured by SLEDAI).[60] These observations are inconsistent with an autoimmunogenic role for APRIL. Indeed, given the ability of APRIL to bind to TACI and potentially deliver a *negative* signal to B cells, it may be therapeutically judicious to choose a BLyS antagonist that does *not* also antagonize APRIL.

Are all SLE patients candidates for BLyS antagonist therapy?

Regardless of the specific agent or the specific modality one wishes to use, the answer to the question above depends upon how one views the role of BLyS in SLE. In principle, BLyS may assume at least two distinct functions as it pertains to SLE. On the one hand, BLyS may serve as a *contributor* to development of SLE. BLyS *per se* may not cause loss of tolerance to self-antigens, but once such tolerance is broken, the ever present nature of the autoantigen permits it to repetitively stimulate the host immune system and elicit a detectable autoimmune response. In the presence of increasing amounts of BLyS, the autoimmune response is exaggerated. In the presence of additional permissive genetic and/or environmental factors, this exaggerated autoimmune response can lead to frank clinical disease.

Accordingly, a reduction in SLE contributory BLyS levels to 'normal' should ameliorate disease by suppressing the BLyS driven acceleration or exaggeration of the autoimmune response. Self-tolerance would remain 'lost', and the autoimmune response would persist. Of critical therapeutic importance, however, the *magnitude* of the autoimmune response would be now insufficient to drive *clinical disease*. Thus, SLE patients with the most elevated circulating BLyS levels should be the ones most responsive to BLyS antagonist therapy. Those patients with normal circulating BLyS levels might be relatively resistant to BLyS antagonist therapy, since 'excess' BLyS in these patients would not be driving the *clinical* autoimmunity.

On the other hand, BLyS may serve as a passive *facilitator* in development of SLE. In this model, development of the pathologic anti-self response is inherently BLyS *independent*. Regardless of whether BLyS levels are normal or elevated, the magnitude of the autoimmune response is similar. That is, the trigger of autoimmunity elicits a response so robust that it is not further amplified by elevated levels of BLyS. Indeed, the fact that a substantial number of SLE

patients do not manifest signs of BLyS overexpression (i.e., they harbor normal serum BLyS and blood BLyS mRNA levels and surface express normal levels of BLyS on their peripheral blood mononuclear cells)[47] strongly suggests that BLyS overexpression is *not* absolutely essential to development of SLE. Nevertheless, given the indispensable role for BLyS in B cell development,[36,37] a certain *threshold level* of BLyS is required to permit *any* antibody responses (including *autoantibody* responses). When BLyS levels are reduced below this critical threshold level, the ability to fully mount an autoimmune response (along with other B cell and humoral responses) is impaired. Accordingly, the SLE patients that should be most responsive to BLyS antagonist therapy should be those with *normal*, rather than elevated, circulating BLyS levels, since in such patients, less neutralization of BLyS would be required to reach the critical threshold level.

These two models are not necessarily mutually exclusive. Within the human SLE population, there may be individuals in whom BLyS plays more of a *contributor* role, and there may be others in whom BLyS plays more of a *facilitator* role. Indeed, from a therapeutic perspective, the models may operationally be viewed as a continuum, with some patients requiring more neutralization of BLyS than that required by others before salutary clinical effects can be appreciated.

Closing remarks

Based on very compelling *in vivo* studies in mice and *ex vivo* studies in humans, BLyS likely contributes to and/or facilitates SLE pathogenesis. Due to the highly restricted nature of its cellular targets (i.e., B cells), specific BLyS antagonists have an excellent chance of not promoting multiple global toxicities. Clinical efficacy of BLyS antagonists has been unequivocally shown in mice but still remains to be formally proven in humans. Although much additional investigation is necessary, one can be cautiously optimistic that specific BLyS antagonists will become important and valuable therapeutic tools in the management of patients with SLE.

References

1 Moore PA, Belvedere O, Orr A *et al.* BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 1999; **285**: 260–263.
2 Shu H-B, Hu W-H, Johnson H. TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. *J Leukocyte Biol* 1999; **65**: 680–683.
3 Schneider P, MacKay F, Steiner V *et al.* BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999; **189**: 1747–1756.
4 Mukhopadhyay A, Ni J, Zhai Y, Yu G-L, Aggarwal BB. Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor-κB, and c-Jun NH_2-terminal kinase. *J Biol Chem* 1999; **274**: 15978–15981.
5 Tribouley C, Wallroth M, Chan V *et al.* Characterization of a new member of the TNF family expressed on antigen presenting cells. *Biol Chem* 1999; **380**: 1443–1447.
6 Gross JA, Johnston J, Mudri S *et al.* TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* 2000; **404**: 995–999.
7 Nardelli B, Belvedere O, Roschke V *et al.* Synthesis and release of B-lymphocyte stimulator from myeloid cells. *Blood* 2001; **97**: 198–204.
8 Kanakaraj P, Migone T-S, Nardelli B *et al.* BLyS binds to B cells with high affinity and induces activation of the transcription factors NF-κB and ELF-1. *Cytokine* 2001; **13**: 25–31.
9 Liu Y, Xu L, Opalka N, Kappler M, Shu H-B, Zhang G. Crystal structure of sTALL-1 reveals a virus-like assembly of TNF family ligands. *Cell* 2002; **108**: 383–394.
10 Kim HM, Yu KS, Lee ME *et al.* Crystal structure of the BAFF-BAFF-R complex and its implications for receptor activation. *Nat Struct Biol* 2003; **10**: 342–348.
11 Liu Y, Hong X, Kappler J *et al.* Ligand-receptor binding revealed by the TNF family member TALL-1. *Nature* 2003; **423**: 49–56.
12 Karpusas M, Cachero TG, Qian F *et al.* Crystal structure of extracellular human BAFF, a TNF family member that stimulates B lymphocytes. *J Mol Biol* 2002; **315**: 1145–1154.
13 Oren DA, Li Y, Volovik Y *et al.* Structural basis of BLyS receptor recognition. *Nat Struct Biol* 2002; **9**: 288–292.
14 Scapini P, Nardelli B, Nadali G *et al.* G-CSF-stimulated neutrophils are a prominent source of functional BLyS. *J Exp Med* 2003; **197**: 297–302.
15 Litinskiy MB, Nardelli B, Hilbert DM *et al.* DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol* 2002; **3**: 822–829.
16 Laabi Y, Gras M-P, Brouet J-C, Berger R, Larsen C-J, Tsapis A. The BCMA gene, preferentially expressed during B lymphoid maturation, is bidirectionally transcribed. *Nucleic Acids Res* 1994; **22**: 1147–1154.
17 von Bülow G-U, Bran RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. *Science* 1997; **278**: 138–141.
18 Thompson JS, Bixler SA, Qian F *et al.* BAFF-R, a novel TNF receptor that specifically interacts with BAFF. *Science* 2001; **293**: 2108–2111.
19 Yan M, Brady JR, Chan B *et al.* Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. *Curr Biol* 2001; **11**: 1547–1552.
20 Xia X-Z, Treanor J, Senaldi G *et al.* TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. *J Exp Med* 2000; **192**: 137–143.
21 Novak AJ, Darce JR, Arendt BK *et al.* Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood* 2004; **103**: 689–694.
22 Avery DT, Kalled SL, Ellyard JI *et al.* BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J Clin Invest* 2003; **112**: 286–297.
23 Shu H-B, Johnson H. B cell maturation protein is a receptor for the tumor necrosis factor family member TALL-1. *Proc Natl Acad Sci USA* 2000; **97**: 9156–9161.
24 Hatzoglou A, Roussel J, Bourgeade M-F *et al.* TNF receptor family member BCMA (B cell maturation) associates with TNF receptor-associated factor (TRAF) 1, TRAF2, and TRAF3 and activates NF-κB, Elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. *J Immunol* 2000; **165**: 1322–1330.
25 Xu L-G, Shu H-B. TNFR-associated factor-3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF-κB activation and IL-10 production. *J Immunol* 2002; **169**: 6883–6889.
26 Hikida M, Johmura S, Hashimoto A, Takezaki M, Kurosaki T. Coupling between B cell receptor and phospholipase C-γ2 is essential for mature B cell development. *J Exp Med* 2003; **198**: 581–589.

27 Claudio E, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NK-κB2 in maturing B cells. *Nat Immunol* 2002; **3**: 958–965.
28 Kayagàki N, Yan M, Seshasayee D *et al.* BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-κB2. *Immunity* 2002; **10**: 515–524.
29 Hatada EN, Do RKG, Orlofsky A *et al.* NF-κB1 p50 is required for BLyS attentuation of apoptosis but dispensible for processing of NF-κB2 p100 to p52 in quiescent mature B cells. *J Immunol* 2003; **171**: 761–768.
30 Thompson JS, Schneider P, Kalled SL *et al.* BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. *J Exp Med* 2000; **192**: 129–135.
31 Do RKG, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. *J Exp Med* 2000; **192**: 953–964.
32 Batten M, Groom J, Cachero TG *et al.* BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med* 2000; **192**: 1453–1465.
33 Harless SM, Lentz VM, Sah AP *et al.* Competition for BLyS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers. *Curr Biol* 2001; **11**: 1986–1989.
34 Hsu BL, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BLyS enables survival of transitional and mature B cells through distinct mediators. *J Immunol* 2002; **168**: 5993–5996.
35 Amanna IJ, Dingwall JP, Hayes CE. Enforced *bcl-x*$_L$ gene expression restored splenic B lymphocyte development in BAFF-R mutant mice. *J Immunol* 2003; **170**: 4593–4600.
36 Gross JA, Dillon SR, Mudri S *et al.* TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLyS. *Immunity* 2001; **15**: 289–302.
37 Schiemann B, Gommerman JL, Vora K *et al.* An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 2001; **293**: 2111–2114.
38 Xu S, Lam D-P. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensible for humoral immune responses. *Mol Cell Biol* 2001; **21**: 4067–4074.
39 von Bülow G-U, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. *Immunity* 2001; **14**: 573–582.
40 Yan M, Wang H, Chan B *et al.* Activation and accumulation of B cells in TACI-deficient mice. *Nat Immunol* 2001; **2**: 638–643.
41 Seshasayee D, Valdez P, Yan M, Dixit VM, Tumas D, Grewal IS. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. *Immunity* 2003; **18**: 279–288.
42 Hahn BH, Tsao BP. Antibodies to DNA. In Wallace DJ, Hahn BH, eds *Dubois' lupus erythematosus*. 5th edition. Baltimore: Williams & Wilkins, 1997: 407–422.
43 Mackay F, Woodcock SA, Lawton P *et al.* Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 1999; **190**: 1697–1710.
44 Khare SD, Sarosi I, Xia X-Z *et al.* Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc Natl Acad Sci USA* 2000; **97**: 3370–3375.
45 Zhang J, Roschke V, Baker KP *et al.* Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. *J Immunol* 2001; **166**: 6–10.
46 Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis Rheum* 2001; **44**: 1313–1319.
47 Stohl W, Metyas S, Tan S-M *et al.* B lymphocyte stimulator (BLyS) overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum* 2003; **48**: 3475–3486.
48 Petri M, Stohl W, Chatham W *et al.* BLyS plasma concentrations correlate with disease activity and levels of anti-dsDNA autoantibodies and immunoglobulins (Ig) in a SLE patient observational study. *Arthritis Rheum* 2003; **48**: S655.
49 Baker KP, Edwards BM, Main SH *et al.* Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes BLyS bioactivities. *Arthritis Rheum* 2003; **48**: 3253–3265.
50 Furie R, Stohl W, Ginzler E *et al.* Safety, pharmacokinetic and pharmacodynamic results of a phase 1 single and double dose-escalation study of Lymphostat-B (human monoclonal antibody to BLyS) in SLE patients. *Arthritis Rheum* 2003; **48**: S377.
51 Gavin AL, Aït-Azzouzene D, Ware CF, Nemazee D. ΔBAFF, an alternate splice isoform that regulates receptor binding and biopresentation of the B cell survival cytokine, BAFF. *J Biol Chem* 2003; **278**: 38220–38228.
52 Hahne M, Kataoka T, Schröter M *et al.* APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. *J Exp Med* 1998; **188**: 1185–1190.
53 Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A. Interaction of the TNF homologues BLyS and APRIL with the receptor homologues BCMA and TACI. *Curr Biol* 2000; **10**: 785–788.
54 Yu G, Boone T, Delaney J *et al.* APRIL and TALL-1 and receptors BCMA and TACI: system for regulating humoral immunity. *Nat Immunol* 2000; **1**: 252–256.
55 Wu Y, Bressette D, Carrell JA *et al.* Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLyS. *J Biol Chem* 2000; **275**: 35478–35485.
56 Rennert P, Schneider P, Cachero TG *et al.* A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. *J Exp Med* 2000; **192**: 1677–1683.
57 Craxton A, Magaletti D, Ryan EJ, Clark EA. Macrophage- and dendritic cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. *Blood* 2003; **101**: 4464–4471.
58 Stein JV, López-Fraga M, Elustondo FA *et al.* APRIL modulates B and T cell immunity. *J Clin Invest* 2002; **109**: 1587–1598.
59 Roschke V, Sosnovtseva S, Ward CD *et al.* BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. *J Immunol* 2002; **169**: 4314–4321.
60 Stohl W, Metyas S, Tan S-M *et al.* Inverse association between circulating APRIL levels and serologic and clinical disease activity in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2004; In press.